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Published in:
Animal Behavior

DOI:
10.1016/j.anbehav.2008.10.029

Publication date:
2009

Animal Behaviour 77 (2009) 581–593

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/yanbe

Does the ring species concept predict vocal variation in the crimson rosella, *Platycercus elegans*, complex?

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ARTICLE INFO

Article history:
Received 16 April 2008
Initial acceptance 5 May 2008
Final acceptance 12 October 2008
Published online 31 December 2008
MS. number: 08-00241R

Keywords:
biogeography
contact call
crimson rosella complex
parrot
Platycercus elegans
ring species
speciation
vocalization

Vocal variation may be important in population divergence. We studied geographical variation in contact calls of parrots of the crimson rosella, *Platycercus elegans*, complex, which is characterized by striking geographical plumage coloration variation. This complex has long been considered a rare example of a ring species (where two divergent forms coexist in sympatry but are connected by a chain of intermediate populations forming a geographical ring). We tested whether contact call variation is consistent with the ring species hypothesis. We recorded calls throughout the ring, including several sites from the three main population groups forming the ring and interfaces between them. We analysed duration, peak frequency, fundamental frequency and frequency modulation. We found significant differences, particularly in fundamental frequency and frequency modulation, at multiple biogeographical scales ranging from local populations to subspecies level. Discriminant function analyses showed some populations could be reliably discriminated from call structure. However, our results provided little support for three key predictions of the ring species hypothesis: (1) calls of the terminal, most divergent forms were not significantly different in three of the four acoustic variables, and differences did not appear to be maintained in sympatry, (2) phenotypically/geographically intermediate populations were not characterized by intermediate calls, and (3) call variation was not concordant with geographical sequence around the ring from one terminal form to the other. Our results underscore the emerging view that the evolutionary histories and phenotypic variability of many long-held ring species may be inadequately described by the ring species hypothesis and require alternative explanations.

In birds, divergence in prezygotic isolating mechanisms is considered one of the causes of speciation (Price & Bouvier 2002; Edwards et al. 2005). Such mechanisms usually rely on variation in characters that allow population or species recognition, such as plumage coloration, morphology or behavioural traits (e.g. Price 1998; Edwards et al. 2005). Divergence is thought to result from selection or, to a lesser extent, from drift as a result of geographical or ecological barriers (Endler 1977; Coyne & Orr 2004; Price 2008). Culturally transmitted behavioural characters are of special interest, as they could diverge rapidly. Such characters are invariably learned and may withstand the homogenizing effects of gene flow (e.g. Wright & Wilkinson 2001; Wright et al. 2005; Leader et al. 2008). Thus, they may precede and promote population divergence in more slowly evolving morphological traits by generating reproductive isolation upon secondary contact (Slabbekoorn & Smith 2002a; Edwards et al. 2005; but see Ellers & Slabbekoorn 2003; Seddon & Tobias 2007; Seddon et al. 2008). In many bird species, vocal signals are likely to be important isolating mechanisms (e.g. Edwards et al. 2005). This is due to their central role in mate choice and species recognition (e.g. Catchpole & Slater 1995), as well as the large number of variables thought to modulate vocal variation and rapid cultural evolution in vocal learners such as oscine songbirds and parrots (Marler 1960; Searcy & Anderson 1986; Catchpole 1987; Grant & Grant 1996; Searcy & Yasukawa 1996; Edwards et al. 2005).

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0003-3472/$38.00
doi:10.1016/j.anbehav.2008.10.029

Numerous factors have been proposed to result in vocal variation between populations of single or closely related species (reviewed in Slabbekoorn & Smith 2002a; Podos et al. 2004; Edwards et al. 2005), but the main factors underlying vocal variation are often unclear (Irwin 2000). Among those that are often considered important are: (1) ecological selection, either directly as a result of habitat features that affect sound transmission (the 'acoustic adaptation hypothesis', Morton 1975; see also Wiley & Richards 1983; Endler 1992; Forrest 1994; Jones 1997; Patten et al. 2004; Seddon 2005; Nicholls et al. 2006) or indirectly because of morphological adaptations to different environments that affect sound production (Slabbekoorn & Smith 2000, 2002b; Podos 2001; Seddon 2005; Ballantine 2006), (2) sympatric interactions such as character displacement and reinforcement (Dobzhansky 1940; Howard 1993; Haavie et al. 2004; Seddon 2005), and (3) cultural (Wiens 1982; Grant & Grant 1997; Grant et al. 2000) or genetic drift (Baker et al. 1982; MacDougall-Shackleton & MacDougall-Shackleton 2001; but see Wright et al. 2005) caused by reduced dispersal or gene flow between geographically or ecologically separated populations. Conversely, in sympatric or parapatric populations gene flow and cultural effects arising from dispersal may simultaneously hinder vocal divergence (Slabbekoorn & Smith 2002a). Such cultural effects are particularly likely in species with open-ended (i.e. lifelong) learning such as parrots, where immigrants may have a tendency to adopt the calls of a new area to facilitate social interactions (Wright 1996). However, many recent studies incorporating molecular data have shown that geographical variation in vocalizations is sometimes maintained even when there appears to be considerable ongoing gene flow (e.g. Soha et al. 2004; Wright et al. 2005; Nicholls et al. 2006; Irwin et al. 2008). This makes vocalizations especially interesting for their potential role in parapatric and sympatric speciation (Sorenson et al. 2003; Edwards et al. 2005; Balakrishnan & Sorenson 2006).

Excellent opportunities to address the relationships between vocal variation and population divergence are afforded by rare cases of 'ring species', or 'circular overlaps' (reviewed in Irwin et al. 2001a; Irwin & Irwin 2002; Martens & Päckert 2007). An ideal ring species would comprise two distinct forms, which coexist in sympatry with little or no gene flow between them but which are connected by a chain of intergrading, intermediate populations that continuously encircle a geographical barrier (Mayr 1942, 1963; Endler 1977). They are suggested to form when an ancestral population expands around a geographical barrier, culminating in secondary contact between the terminal forms. Divergence is thought to arise in the face of gene flow through isolation by distance (Irwin et al. 2005), rather than through historical allopatry. Thus, the ring species hypothesis makes several predictions about how traits should vary geographically. The main value of ring species lies in the potential to trace how gradual geographical variation in traits around the ring may lead to population divergence and speciation. Only about 23 are proposed worldwide across all taxa, and many of these appear not to fulfil all of the criteria of an ideal ring species indicated above (Irwin et al. 2001a). In several of the proposed cases of avian ring species, some variation in both vocalizations and plumage coloration has been reported between the different populations, but few cases have been studied in detail (Irwin et al. 2001a).

We studied vocal variation among populations of the crimson rosella, *Platycercus elegans*, complex, which has long been considered a ring species encircling unsuitable, arid habitat in southeastern Australia (Fig. 1a; Cain 1955; Mayr 1963; Irwin & Irwin 2002). Currently, the main distinguishing features of this near-complete 'ring' are thought to be plumage coloration on the dorsal and ventral surfaces of the birds and, to a lesser extent, body size (e.g. Joseph & Hope 1984; Ovenden et al. 1987; Higgins 1999; Forshaw 2002). The two terminal forms are the deep red crimson rosella (CR, *P. e. elegans*) and the slightly smaller yellow rosella (YR, *P. e. flaveolus*), which form the southern and northern edges of the ring, respectively. A phenotypically intermediate series of populations collectively referred to as the Adelaide rosella (AR) form the western edge of the ring. AR varies clinally, with redder populations in the south, close to the western extremity of CR's range, becoming progressively more yellow in the north and east, where it approaches the western extremity of YR's range. CR does not currently meet AR, which is isolated by approximately 10 km of sea from one population of CR and by 200 km of unsuitable habitat from another population of CR. However, genetic analyses indicate that these populations retain close genetic relationships (for details see Joseph et al. 2008). Where CR and YR populations meet on the western slopes of the Great Dividing Range in southeastern New South Wales (the eastern edge of the ring), a narrow zone of phenotypically intermediate birds co-occur with both CR and YR phenotypes (hereafter WS; Schodde & Mason 1997; Forshaw 2002; Fig. 1a).

We examined whether the ring species hypothesis accurately predicts geographical contact call variation across populations of the *P. elegans* ring. When applied to *P. elegans* vocalizations, the ideal ring species hypothesis predicts that: (1) CR and YR should have the most distinct calls, which should be maintained where they occur in sympatry (the western slopes region), (2) AR calls should be intermediate between those of CR and YR, and (3) acoustic population differences should vary clinally, reflecting their geographical sequence from the terminal CR to YR populations. To test these predictions using vocalizations, we assessed the extent of contact call variation in members of the *P. elegans* ring at three biogeographical levels of decreasing scale: (1) the four broad groups described above that constitute the entire ring, (2) geographically related zones comprising several local populations within the variable AR, and (3) restricted sites representing local populations.

METHODS

Study Sites

We recorded individuals from 28 sites throughout the range of the *P. elegans* ring species in southeastern Australia, representing AR (*P. e. adelaidae*), CR (*P. e. elegans*), YR (*P. e. flaveolus*) and WS (*P. e. elegans* and *P. e. flaveolus* contact zone) populations. We divided the range into eight 'zones' (Fig. 1) based on plumage colour and geographical location. Within each zone, a mean $\pm$ SE of 3.6 ± 1.8 (range 1–6) recording sites were chosen haphazardly (i.e. randomly from a map, within constraints imposed by road access, property ownership and the presence of rosella habitat). This was to ensure that differences detected between zones were genuine and not a result of random differences between local populations (sites). All sites within a zone were at least 10 km apart, which should virtually eliminate the chance of recording the same individual in different sites, as banding studies suggest adult rosellas rarely, if ever, travel further than 10 km (Higgins 1999; R. Sinclair & P. Bird, personal communication).

Five zones were located in *Eucalyptus* spp. wooded habitat occupied by rosellas throughout the Mount Lofty Ranges and southern Flinders Ranges (South Australia). These form an interconnected series of low ranges running north–south along the eastern shores of Spencer Gulf and the Gulf of St Vincent (Fig. 1a), and the habitat ranges from closed woodland in the south to open woodland and shrubland in the north and east (e.g. Costermans 1984). These zones represent the range of AR, which we categorized according to the three previously recognized taxonomic divisions and differences in plumage colour (Higgins 1999; Forshaw 2002; own unpublished data). Zone 1 (Fleurieu Peninsula, 138°11′S,

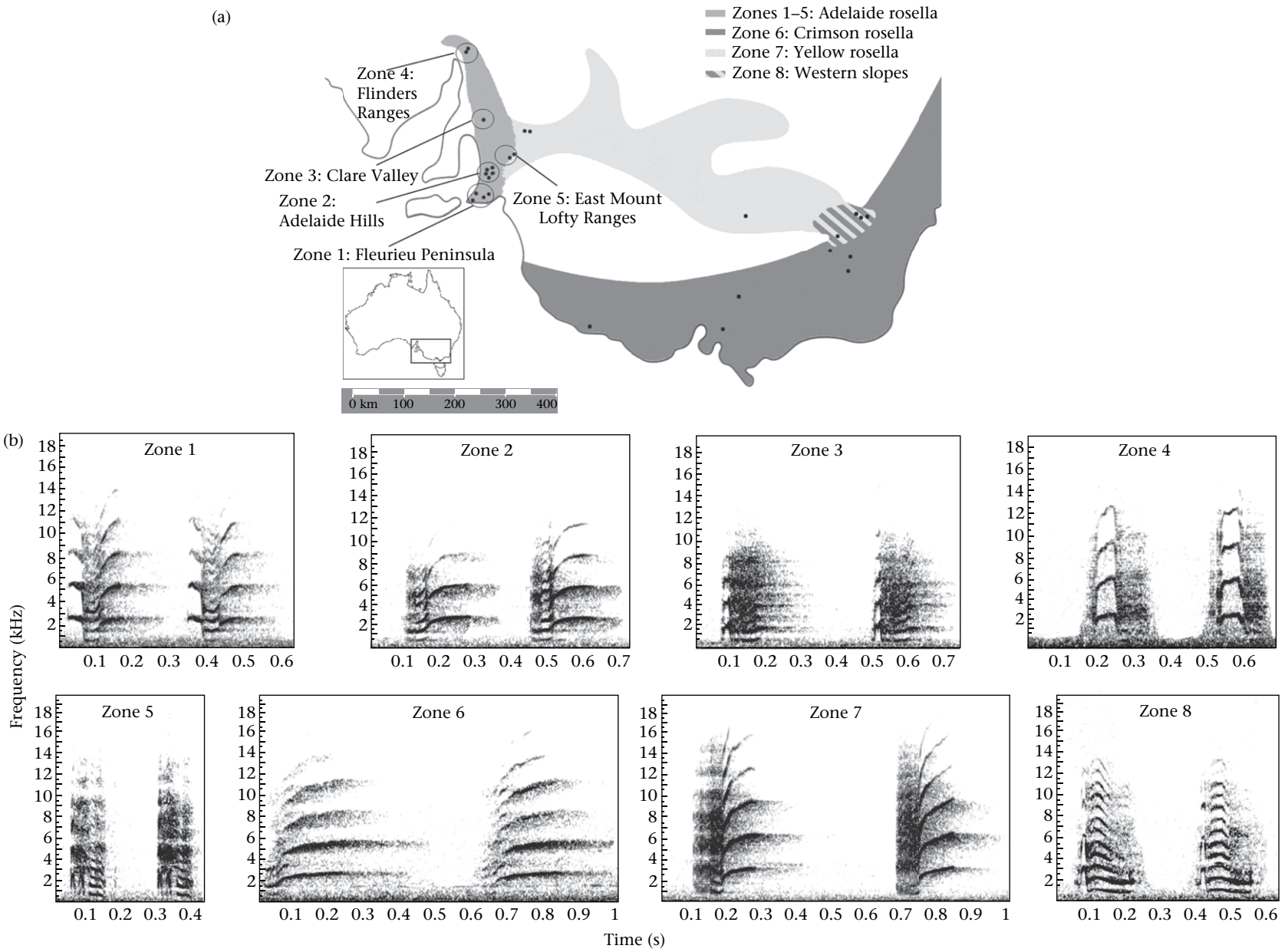

Figure 1. (a) Distribution of the *Platycercus elegans* ring in southeastern Australia, indicating the approximate locations of recording sites (closed circles) and zones (open circles; regions of morphologically similar birds) used for analysis in this study. The shaded areas indicate the distributions of the morphologically distinct Adelaide rosella, crimson rosella, yellow rosella, and western slopes populations where crimson rosella and yellow rosella occur in sympatry. (b) Spectrograms show a representative contact call couplet from each sampling zone. Map modified from Forshaw (2002).

35°37′E, 7 recording days from 17 September to 28 November 2004) comprised red birds (*P. e. fleurieuensis*) occurring in the southern part of the Mount Lofty Ranges. Zone 2 (Adelaide Hills, 138°44′S, 34°58′E, *P. e. adelaidae*, 16 recording days from 21 September 2004 to 22 January 2005) consisted of predominantly orange birds in the central, highest parts of the Mount Lofty Ranges. Zones 3 (Clare Valley, 138°35′S, 33°53′E, *P. e. subadelaidae* meets *P. e. adelaidae*, 2 recording days from 9 October to 13 November 2004) and 4 (Flinders Ranges, 138°01′S, 32°11′E, *P. e. subadelaidae*, 2 recording days, 12 and 13 November 2004) encompassed progressively more yellow birds of open woodlands of the northern Mount Lofty Ranges and southern Flinders Ranges. Zone 5 (East Mount Lofty Ranges, 139°18′S, 34°39′E, *P. e. adelaidae*, 4 recording days from 21 November 2004 to 15 January 2005) was located in open woodland on riverine plains at the eastern border of the range of AR, where it approaches YR (although possibly not in current contact; Joseph & Hope 1984; Forshaw 2002).

Zone 6 represents CR (crimson rosella, *P. e. elegans*, 9 recording days from 3 November 2004 to 21 January 2005), which occurs extensively in wet forest and woodland habitats in southeastern and eastern Australia. These sites comprised Crawford River National Park (141°27′S, 37°57′E) at the western end of the CR range, where most recordings took place, and several sites around Melbourne (145°39′S, 37°58′E) and northeastern Victoria (146°59′S, 36°18′E). The YR (yellow rosella, *P. e. flaveolus*, zone 7, 6 recording days from 9 November 2004 to 19 January 2005) occurs only in inland riparian open woodland (Higgins 1999; Forshaw 2002). Recordings of YR were made at sites along the Murray River at Cadell (139°45′S, 34°03′E) and Barmah State Forest (145°15′S, 35°51′E).

Zone 8 represents populations where CR and YR meet in the western slopes region. We recorded these birds around Albury–Wodonga (146°55′S, 36°09′E) and Gundagai, NSW (148°05′S, 35°04′E, 14 recording days from 23 October to 19 December 2004). This zone comprised individuals that were phenotypically similar to CR or YR, along with a large proportion of phenotypically intermediate individuals.

This research was conducted with the approval of the South Australian Wildlife Ethics Committee, the NSW Department of Primary Industries Animal Care and Ethics Committee and a Victorian research permit.

Vocal Recordings: Contact Calls

Three call types are most characteristic of the vocal repertoire of rosellas: a contact call, a 'piping' call, of unclear function but thought to be involved in individual identification, and a 'chattering' call, most often used in the context of aggressive interactions (Higgins 1999; Forshaw 2002; R. Astley, unpublished data). We focused on the contact call, as it is the most common call encountered, it offers an acoustic structure amenable to detailed analysis, and it appears to be acoustically and functionally most similar to the call types considered in several previous studies of parrot vocalizations (e.g. Wright 1996; Baker 2000; Bradbury et al. 2001). The main function of the parrot contact call is thought to be maintaining contact between pair and group members and supporting social activities (Saunders 1983; Farabaugh et al. 1994; Wright 1996; Bradbury 2003). There are indications that individuals can recognize contact calls from conspecifics, even on an individual level (Wanker et al. 2005). Similarity in contact calls plays an important role in the mate choice of another Australian parrot, the budgerigar, *Melopsittacus undulatus* (Hile et al. 2000; Moravec et al. 2006). Moravec et al. (2006) presented evidence that budgerigars paired assortatively based on the prepairing similarity of their contact calls, and that paternal care was also positively correlated with prepairing call similarity. Thus, geographical variation in contact calls may hinder dispersal and promote reproductive isolation between populations, because immigrants to new populations are less successful at establishing a territory, pairing, raising offspring or assimilating into social groups (but see Wright & Wilkinson 2001; Wright et al. 2005).

Calls were recorded from free-living birds ($N = 220$) during the main breeding season, from September to January, during daylight hours. Birds were either flying ($N = 129$) or perching ($N = 91$) when recorded. Once an individual was recorded, the next recording would be taken as soon as possible, at least 500 m away (unless individuals were individually identifiable); in this way, we endeavoured to ensure that we were recording from different individuals within each site. The approximate location (±5 m) of each recording was recorded with a global positioning system receiver (Garmin eTrex, Kansas City, KS, U.S.A.). The recordings were made with a solid state digital sound recorder (Marantz PMD670) and directional microphone (Sennheiser ME64/K6) mounted on a parabolic wind shield (Telinga, Tobo, Sweden). Calls were recorded at 16-bit with a 44.1 kHz sampling rate. At the time of recording, we tried to observe the calling bird through binoculars which could then be classified as first year or older than 1 year based on the presence of green breast and rump plumage (Higgins 1999; Forshaw 2002). Juveniles, and calls that could not be unequivocally assigned to a particular individual, were excluded from the analyses.

Acoustic Analyses

Sound files (uncompressed wav format) were transferred to the computer for analysis with SASLab Pro version 4.36 (Avisoft, Berlin, Germany), Syrinx-PC version 2.4s (J. Burt, syrinxpc.com) and Sound Analysis Pro version 1.04 (Tchernichovski et al. 2004). Because rosella flight calls are often delivered as a couplet (Higgins 1999), two successive contact calls for each individual were selected at random ($N = 213$ individuals, 86.7%) from the original recording whenever available, and filtered to remove noise under 0.5 kHz using SASLab Pro. We only included recordings in the analyses that were of sufficient clarity and amplitude to digitize and unequivocally score the acoustic variables described below. Recordings were also taken only when the birds were not producing alarm calls (Higgins 1999), and had no other sounds interfering in the background.

We examined four acoustic variables that capture spectral and temporal characteristics of rosella contact calls and that we identified a priori as having potential importance to parrot communication: (1) call duration (Baker 2000; Bradbury et al. 2001), (2) peak frequency (Farabaugh et al. 1998; Wright & Wilkinson 2001; Wright et al. 2003), (3) fundamental frequency (Baker & Logue 2003), and (4) mean frequency modulation (FM; Baker & Logue 2003). Duration was measured with on-screen cursors in SASLab Pro, using standard spectrogram settings. Peak frequency and mean FM were measured automatically by SASLab Pro and Sound Analysis Pro, respectively, using default settings. Spectrograms (Fig. 2b) were used to estimate the fundamental frequency, by measuring the frequency of the lowest harmonic of each syllable (which was equivalent to the interharmonic frequency range). If a consistent fundamental frequency was not maintained throughout the call, the fundamental frequency of the longest section was analysed (e.g. second fundamental frequency in Fig. 2b). In some calls no harmonics were detected and here the fundamental frequency was treated as a missing value. The last variable, mean FM, was calculated by Sound Analysis Pro; mean FM gives an absolute value for the deviation of frequencies, in reference to a constant frequency, for any selected syllable (Tchernichovski et al. 2000; Baker & Logue 2003).

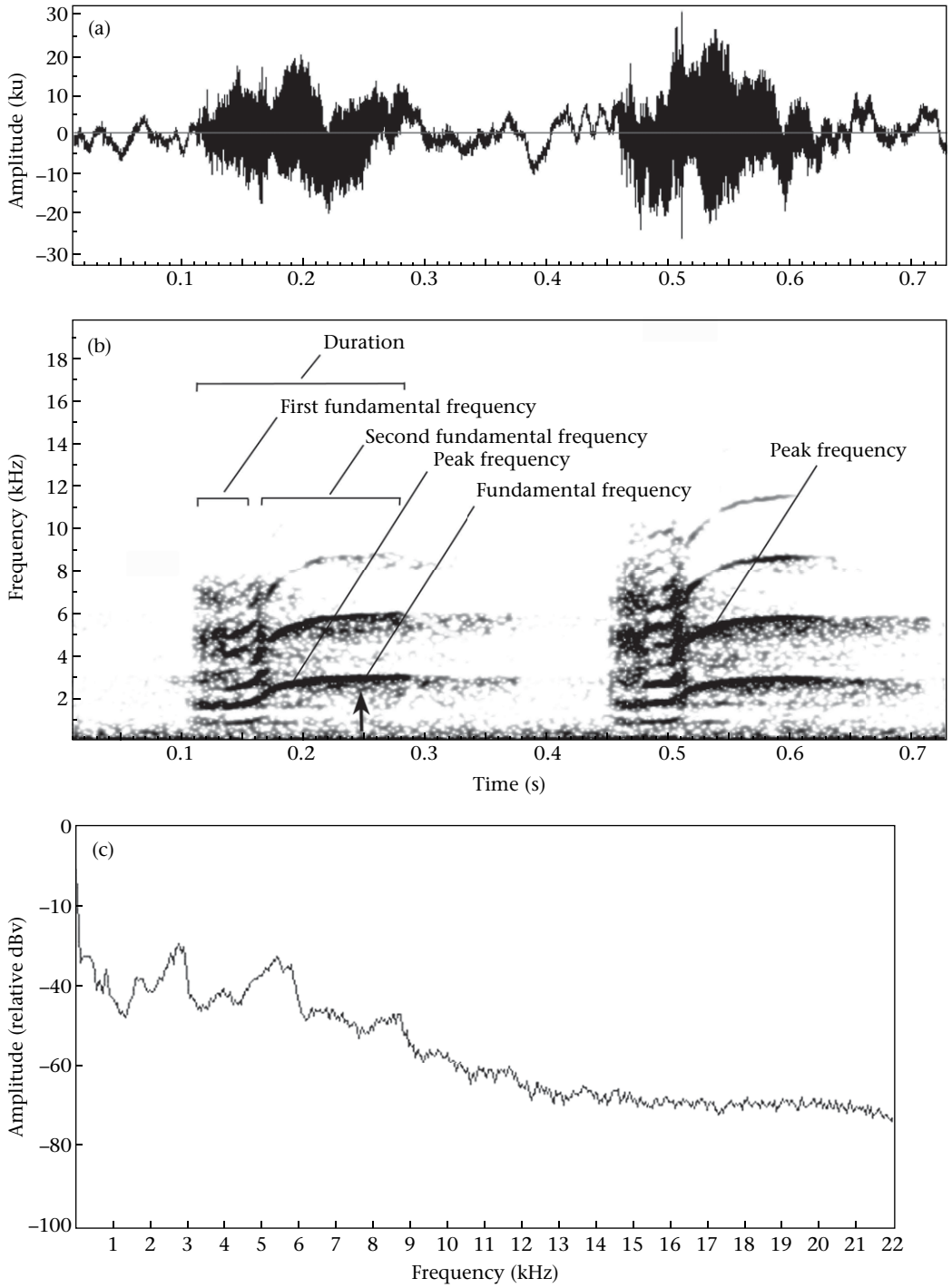

Figure 2. Example (a) waveform, (b) spectrogram and (c) amplitude spectrum of a contact call couplet from an Adelaide rosella, *Platycercus elegans adelaide*, showing the characteristic harmonics. Labels indicate the sections with different fundamental frequencies, and the acoustic variables taken from spectrograms for use in this study (see Methods). The amplitude spectrum shows two peaks (at approximately 2.8 and 5.5 kHz) which correspond to the peak frequencies of the first and second calls, respectively. Spectrograms were created with Syrinx-PC (transform rate/FFT: 512; window type: Blackman; DFT size: 1024; scale: 0–22 kHz; 3 dB filter bandwidth: 141 Hz; overlap: default; J. Burt, www.syrinxpc.com). Amplitude spectra were created with AnalFreq 1.80 (Bossis 2003).

Statistical Analyses

Statistical analyses were performed in SPSS versions 12 and 15 (SPSS Inc., Chicago, IL, U.S.A.), and follow Quinn & Keough (2002) and Tabachnick & Fidell (2007). For each dependent variable (duration, peak frequency, fundamental frequency and mean FM), we constructed a multilevel linear mixed model (proc MIXED) with restricted maximum likelihood (REML) estimation to test for differences in acoustic variables between populations. We used this approach to model the covariance among the measurements taken from individuals (we analysed one to two calls per individual) and among the recording sites within zones. The dependent variables were each analysed separately, to allow full interpretation of the effects of particular acoustic variables and because combining them using a principal components analysis did not allow for reduction in variables (PC1 explained 35.4% variance, PC2 explained 31.4%

variance, and PC3 explained 17.9% variance). With varimax rotation, necessary for adequate interpretation, each PC explained 25.0% variance with eigenvalues of 1.0. Pairwise correlation coefficients (r) between our acoustic variables were uniformly low and ranged from 0.046 to 0.347. Each mixed model tested the effects of zone (fixed effects) and recording site (random effect nested within zone), while controlling for recording date and individual identity (random subject variable in SPSS). In analyses that included CR, YR and WS, we lumped zones 1–5 (AR). We set the repeated covariance type to scaled identity (assumes constant variance and zero covariance), which provided qualitatively similar estimates to a compound symmetry covariance type and provided the best fitting model as assessed by Akaike's information criterion (AIC), and Hurvich & Tsai's information criterion (AICc). Estimates of fixed effects are reported (F statistics and P values) with tests of significance based on type III sums of squares. For pairwise comparisons between groups, we present Bonferroni-corrected significance tests. Where applicable, degrees of freedom are rounded to whole numbers.

In addition to mixed models, we used discriminant function analyses (DFA; proc DISCRIMINANT) to classify individuals by call variables. We conducted direct DFA (all predictors were used) using all available canonical discriminant functions. We used separate covariance matrices for each group to account for heterogeneity of variance–covariance matrices between groups (Box's $M > 62.9$, $P < 0.011$), and prior probabilities based on group sizes to account for the substantial heterogeneity in group size (in particular, the AR group was larger and more dispersed). Effect sizes were calculated as the squared canonical correlation for each discriminant function. Discriminant function loadings of more than 0.5 are reported, but loadings of less than 0.5 are not interpreted. DISCRIMINANT is somewhat less flexible than MIXED (Tabachnick & Fidell 2007) and thus involved the following further considerations. In order not to violate the assumption of independence, we used the mean values for each individual with two calls recorded as the units of analysis, but we treated different individuals within recording sites as independent to maintain sufficient degrees of freedom. Recording date was not included as a covariate, which should make our DFA more conservative. To test for clinal variation around the ring, we ranked zones (except WS) according to their geographical sequence around the ring, and correlated this with the mean acoustic values for each zone. We chose to use the ranked location of zones rather than actual geographical distance around the ring to minimize the potentially confounding effects of range and habitat discontinuities (and the resulting nonlinearity). To test for unimodality (null hypothesis), we used the dip test (Hartigan & Hartigan 1985) implemented in the software package ODDJOB (Dallal 1989). All P values are two-tailed and results were considered significant when $P < 0.05$.

RESULTS

Call Structure

We analysed 409 contact calls from 220 individuals throughout the range. In almost all cases (99%, $N = 405$), these calls consisted of several harmonics often with little amplitude fall-off (Fig. 2). Calls were usually given as a couplet and delivered loudly between members of a pair or group, either in flight or perching. The fundamental frequency of calls almost always (98%, $N = 405$ cases) fell within two distinct frequency ranges, namely 0.8–1.0 kHz or 2.0–3.5 kHz. In some recordings the call consisted of two sections with different fundamental frequencies (52%, $N = 212$ calls). In these cases, the fundamental frequency of each section always fell within either of the two frequency ranges described above, and we chose the longest section for analysis.

Geographical Variation in Calls Throughout the Ring

The vocalizations of AR, CR, YR and WS populations (Fig. 1) differed in the acoustic variables fundamental frequency and mean frequency modulation (FM), but not in call duration or peak frequency (Table 1, Fig. 3). Bonferroni-adjusted post hoc tests revealed that the fundamental frequency of contact calls of WS populations were significantly lower than those of AR, CR and YR populations (Fig. 3c). There were no significant differences in fundamental frequency between the AR, CR and YR populations (mean differences <0.29 kHz, $P > 0.16$). In addition, YR had significantly less mean FM than AR and CR, and tended to have less mean FM than WS (Fig. 3d). However, there were no significant differences between AR, CR and WS in mean FM (mean differences <1.22, $P = 1.000$). As mean FM is a measure of the deviation from a constant frequency throughout the call, this indicates that the contact calls of YR have a more constant frequency than calls from other populations.

We also found significant effects of recording site on all four acoustic variables (Table 1). Intraclass correlations (i.e. within-individual correlations, ρ) were high for fundamental frequency (0.91), moderate for duration and mean FM (0.79 and 0.64, respectively) and low for peak frequency (0.41). Furthermore, recording date showed a significant positive association with call duration, and a near significant positive association with fundamental frequency and mean FM, but the magnitudes of these effects were relatively small (Table 1).

Discriminant function (DF) analysis yielded three dimensions. DF1 accounted for 80.3% of variation ($P < 0.001$, effect size 0.480, eigenvalue 0.925), DF2 accounted for 18.9% ($P < 0.001$, effect size 0.179, eigenvalue 0.218), and DF3 accounted for the remaining variation ($P = 0.349$, effect size 0.010, eigenvalue 0.010). DF1 was positively loaded by fundamental frequency ($r = 0.989$), DF2 was positively loaded by call duration ($r = 0.614$), and DF3 was positively loaded by mean FM ($r = 0.917$) and negatively loaded by call duration ($r = -0.759$). The solution classified 63.3% of individuals into their correct groups overall (Fig. 3e). There was good classification of WS (84.2% correctly classified, the remainder were classified as AR), and moderate classification of AR, with only 61.5% of individuals correctly classified; the remaining AR were classified as CR (12.0%), YR (16.2%) or WS (10.2%). Classification was less successful for both CR (55.3% correctly classified, 36.8% classified as AR and the remainder classified as YR) and YR (52.0% correctly classified, 44.0% classified as AR and the remainder classified as CR). AR was much more variable than the other populations (Fig. 3e). A cross-classification run, with jackknifed classification and pooled variance–covariance matrices, was used to check the stability of the classifications, and resulted in a very low (0.4%) decrease in the overall rate of correct classification (classification of any group changed by $< 2.7\%$).

The ring species hypothesis predicts that differences between CR and YR should be maintained in sympatry (WS populations), resulting in bimodal distributions of trait values, and that traits should vary clinally around the ring. Histograms of acoustic variables from WS populations show that mean FM, the only variable to differ significantly between CR and YR, featured a clearly unimodal distribution in WS populations (dip statistic $= 0.038$, $P > 0.1$; Fig. 4). In contrast, fundamental frequency showed a clearly bimodal distribution in these populations (dip statistic $= 0.164$, $P \leq 0.001$) as a result of the low fundamental frequency of most calls in WS populations relative to calls from CR and YR populations. The geographical sequence of zones 1–7 around the ring was not related to differences in any acoustic variable (Pearson correlations: duration: $r_5 = -0.551$, $P = 0.200$; peak frequency: $r_5 = -0.231$, $P = 0.618$; fundamental frequency: $r_5 = -0.245$, $P = 0.596$; mean FM: $r_5 = 0.072$, $P = 0.879$) or DF (DF1: $r_5 = -0.285$, $P = 0.535$; DF2: $r_5 = -0.385$, $P = 0.393$; DF3: $r_5 = 0.053$,

Table 1
Comparisons of four acoustic variables describing contact calls from five populations of the *Platycercus elegans* ring species complex in southeastern Australia

Explanatory term	Mean	Estimate±SE	*F*	*df*	*P*
Duration (s)					
Population					
AR	0.159±0.006	−0.08±0.035	2.031	3, 186	0.111
CR	0.188±0.012	0.005±0.023			
YR	0.187±0.014	−0.063±0.023			
WS	0.164±0.012				
Site					
AR (14)	0.089–0.224	−0.048–0.087	4.451	24, 186	<0.001
CR (6)	0.146–0.242	−0.076–0.020			
YR (3)	0.155–0.207	0.045–0.052			
WS (5)	0.118–0.217	0.041–0.100			
Date		<0.001±<0.001	4.183	1, 183	0.042
Peak frequency (kHz)					
Population					
AR	3.596±0.138	0.586±0.846	0.975	3, 186	0.406
CR	3.130±0.279	0.465±0.553			
YR	3.310±0.333	0.895±0.545			
WS	3.259±0.298				
Site					
AR (14)	2.717–4.570	−0.817–1.036	2.060	24, 185	0.004
CR (6)	2.171–4.857	−1.241–1.445			
YR (3)	2.710–3.842	0.463–1.132			
WS (5)	2.631–4.481	−0.317–1.533			
Date		0.001±0.004	0.107	1, 80	0.743
Fundamental frequency (kHz)					
Population					
AR	2.428±0.055	1.855±0.31	61.068	3, 189	<0.001
CR	2.701±0.103	1.445±0.201			
YR	2.726±0.122	1.852±0.199			
WS	0.996±0.106				
Site					
AR (14)	0.918–3.059	−1.899–0.241	12.949	24, 189	<0.001
CR (6)	2.340–2.911	−0.067–0.563			
YR (3)	2.642–2.814	0.094–0.172			
WS (5)	0.864–1.331	−0.098–0.369			
Date		0.003±0.001	3.520	1, 187	0.062
Mean frequency modulation					
Population					
AR	36.396±0.644	9.125±3.945	3.897	3, 187	0.010
CR	37.610±1.301	−5.011±2.57			
YR	31.266±1.554	−4.321±2.538			
WS	36.578±1.378				
Site					
AR (14)	27.306–42.456	0.833–15.150	7.336	24, 187	<0.001
CR (6)	28.320–45.508	4.205–17.188			
YR (3)	29.010–34.298	1.488–5.288			
WS (5)	33.041–38.891	−0.289–5.560			
Date		0.034±0.018	3.636	1, 182	0.058

One or two consecutive calls per individual were analysed (see Methods for details). Data were analysed using mixed models, which included the three terms listed and individual identity (and a constant). Means are estimated marginal means for each level of the terms population or site derived from these models. Means and estimates are presented with SE for population, or as a range for the sites within each level of population; the estimate for the last level in each group was excluded and not shown as it is redundant and set to 0. Degrees of freedom are rounded to the nearest whole number. Population categories are Adelaide rosella (AR), crimson rosella (CR), yellow rosella (YR) and western slopes (WS). The number of sites is indicated in parentheses.

$P = 0.911$). These results were qualitatively the same when we excluded the geographically outlying zone 4 (acoustic variables: $-0.534 < r_4 < -0.028$, $P > 0.276$; DFs: $-0.544 < r_4 < -0.358$, $P > 0.264$).

Call Variation Within the Adelaide Rosella

We found significant variation among calls from the five zones (Fig. 1) defined for AR populations. Bonferroni post hoc tests revealed differences between these zones involving all four acoustic variables (Table 2, Fig. 5). Specifically, the East Mount Lofty Ranges zone had shorter calls than the Fleurieu Peninsula, Adelaide Hills and Clare Valley zones (Fig. 5a), a lower fundamental frequency than the Fleurieu Peninsula, Adelaide Hills and Flinders Ranges zones (Fig. 5c), and greater mean FM than the Adelaide Hills zone (Fig. 5d). In addition, the Clare Valley zone had a lower fundamental frequency than the Fleurieu Peninsula, Adelaide Hills and Flinders Ranges zones (Fig. 5c), and lower peak frequency than the Fleurieu Peninsula zone (Fig. 5b). No other significant differences were found between zones for any acoustic variable (call duration: mean differences < 0.05 s, $P > 0.427$; peak frequency: mean differences < 1.13 kHz, $P > 0.109$; fundamental frequency: mean differences < 0.22 kHz, $P = 1.000$; mean FM: mean differences < 6.27, $P > 0.105$).

We found a significant positive effect of recording date on call duration, fundamental frequency and mean FM, although the estimates of these effects were again small (Table 2). Recording site had a significant effect on duration, fundamental frequency and mean FM (Table 2). Intraclass correlations were 0.81 (fundamental frequency), 0.73 (duration), 0.60 (mean FM) and 0.25 (peak frequency).

The five zones of AR varied along four dimensions following DF analysis. DF1 accounted for 91.1% of variation ($P < 0.001$, effect size 0.724, eigenvalue 2.635), DF2 accounted for 7.1% ($P = 0.002$, effect size 0.171, eigenvalue 0.206), DF3 accounted for 1.4% ($P = 0.212$, effect size 0.038, eigenvalue 0.040), and DF4 accounted for the remaining variation ($P = 0.221$, effect size 0.013, eigenvalue 0.013). DF1 was positively loaded by fundamental frequency ($r = 0.993$), DF2 was positively loaded by mean FM ($r = 0.853$) and negatively loaded by call duration ($r = -0.612$), DF3 was negatively loaded by peak frequency ($r = -0.785$), and DF4 was negatively loaded by call duration ($r = -0.766$). The solution classified 74.4% of individuals into their correct groups overall (Fig. 5e). There was good classification of the Adelaide Hills zone (90.0% correctly classified, 5.7% classified as the Fleurieu Peninsula zone and the remainder classified as the Flinders Ranges zone), the East Mount Lofty Ranges zone (75.0% correctly classified, 12.5% classified as the Adelaide Hills zone and the remainder classified as the Clare Valley zone) and the Clare Valley zone (71.4% correctly classified and the remainder classified as the East Mount Lofty Ranges zone). The Fleurieu Peninsula zone was poorly discriminated from the Adelaide Hills zone (46.4% correctly classified, 50.0% classified as the Adelaide Hills zone and the remainder classified as the Clare Valley zone). Furthermore, classification of the Flinders Ranges zone was completely unsuccessful (75.0% classified as the Adelaide Hills zone and the remainder classified as the Fleurieu Peninsula zone). Stability of classification was good, with a cross-classification resulting in a small (4.2%) decrease in the overall rate of correct classification (only classification of the East Mount Lofty Ranges zone changed by >5%, with 25% of individuals being incorrectly classified as the Clare Valley zone in the cross-classification run).

DISCUSSION

We found significant, hitherto undocumented, acoustic variation in contact calls within the *P. elegans* ring, and at all three biogeographical levels under consideration. First, analysis of the contact calls of AR, CR, YR and WS indicates significant differences between these populations in three variables: duration, fundamental frequency and mean FM. Of these four populations, all could be discriminated with more than 50% accuracy on the basis of our acoustic variables, and WS could be discriminated with high

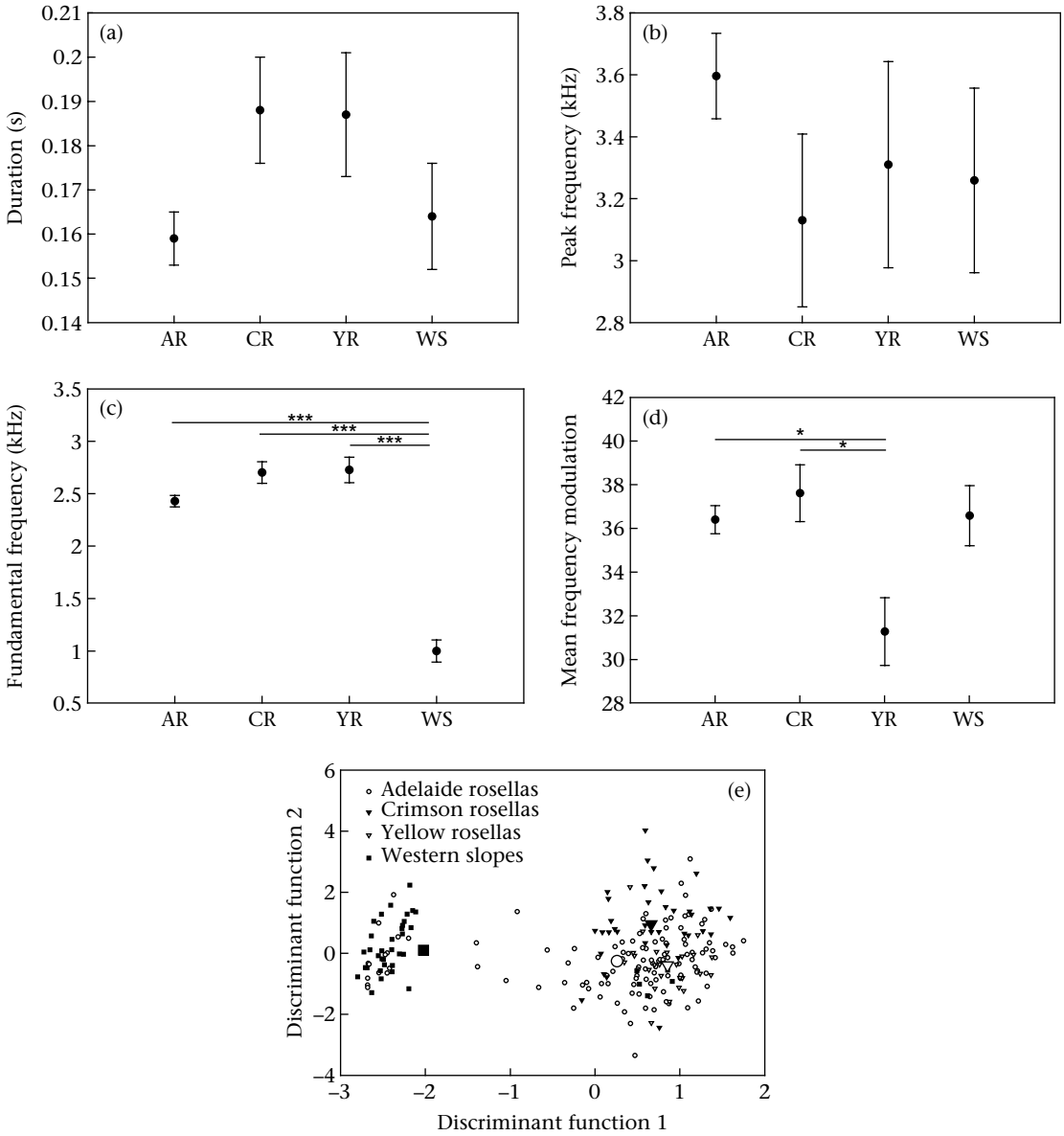

Figure 3. Acoustic variables describing contact calls from four populations of the *Platycercus elegans* ring in southeastern Australia: AR ($N = 119$), CR ($N = 38$), YR ($N = 25$) and WS ($N = 38$). Variables shown are (a) call duration, (b) peak frequency, (c) fundamental frequency and (d) mean frequency modulation (FM). Fundamental frequency of WS populations was significantly lower than AR (mean difference ± SE = −1.43 ± 0.12 kHz (95% confidence interval, CI −1.74 to −1.13), $df = 199$, $P < 0.001$), CR (mean difference ± SE = −1.71 ± 0.15 kHz (95% CI −2.12 to −1.30), $df = 192$, $P < 0.001$) and YR populations (mean difference ± SE = −1.73 ± 0.17 kHz (95% CI −2.17 to −1.29), $df = 191$, $P < 0.001$). YR had significantly lower mean FM than AR (mean difference ± SE = −5.13 ± 1.72 (95% CI −9.73 to −0.54), $df = 180$, $P = 0.020$) and CR (mean difference ± SE = −6.34 ± 1.98 (95% CI −11.62 to −1.07), $df = 179$, $P = 0.090$), and tended to have lower mean FM than WS (mean difference ± SE = −5.31 ± 2.12 (95% CI −10.96 to −0.33), $df = 190$, $P = 0.078$). Bars show mean ± SE of fitted values derived from multilevel models controlling for recording site, date and individual identity (see text and Table 1 for details). $^{*}P < 0.05$; $^{***}P < 0.001$. (e) Discriminant function analysis of contact calls based on the four acoustic variables; each point represents an individual and the four large symbols indicate centroids for the four populations.

accuracy (84% correct classification). Second, duration, peak frequency, fundamental frequency and mean FM were all significantly different between the zones of the Adelaide rosella that we analysed. Among these five zones, one could be discriminated with very high reliability (90% correct classification), and a further two could be discriminated with reasonably high reliability (>70%). Third, we found significant variation in all acoustic variables at the level of local populations (recording site). This was true when considering all populations together, or only AR populations (except regarding peak frequency within AR), and therefore appears to be a robust finding. Discriminability of sites within the Adelaide Hills zone of AR (the largest zone in terms of number of sites) ranged from 66.7% to 71.4% (results not shown).

Support for the Ring Species Hypothesis?

Based on the pattern of acoustic variation observed in contact calls in this study, the predictions of the ring species hypothesis found little support. In particular, CR and YR calls did not differ in duration, peak frequency or fundamental frequency. In no acoustic variables did AR (or WS) clearly appear to be acoustically intermediate between CR and YR. Finally, call variation around the ring did not vary concordantly with geographical sequence; in the cases of mean FM (notable as the only variable to differ significantly between CR and YR) and fundamental frequency, geographical sequence and call similarity between AR populations and CR/YR seemed to be negatively related (Figs 3, 5). Consistent with the ring

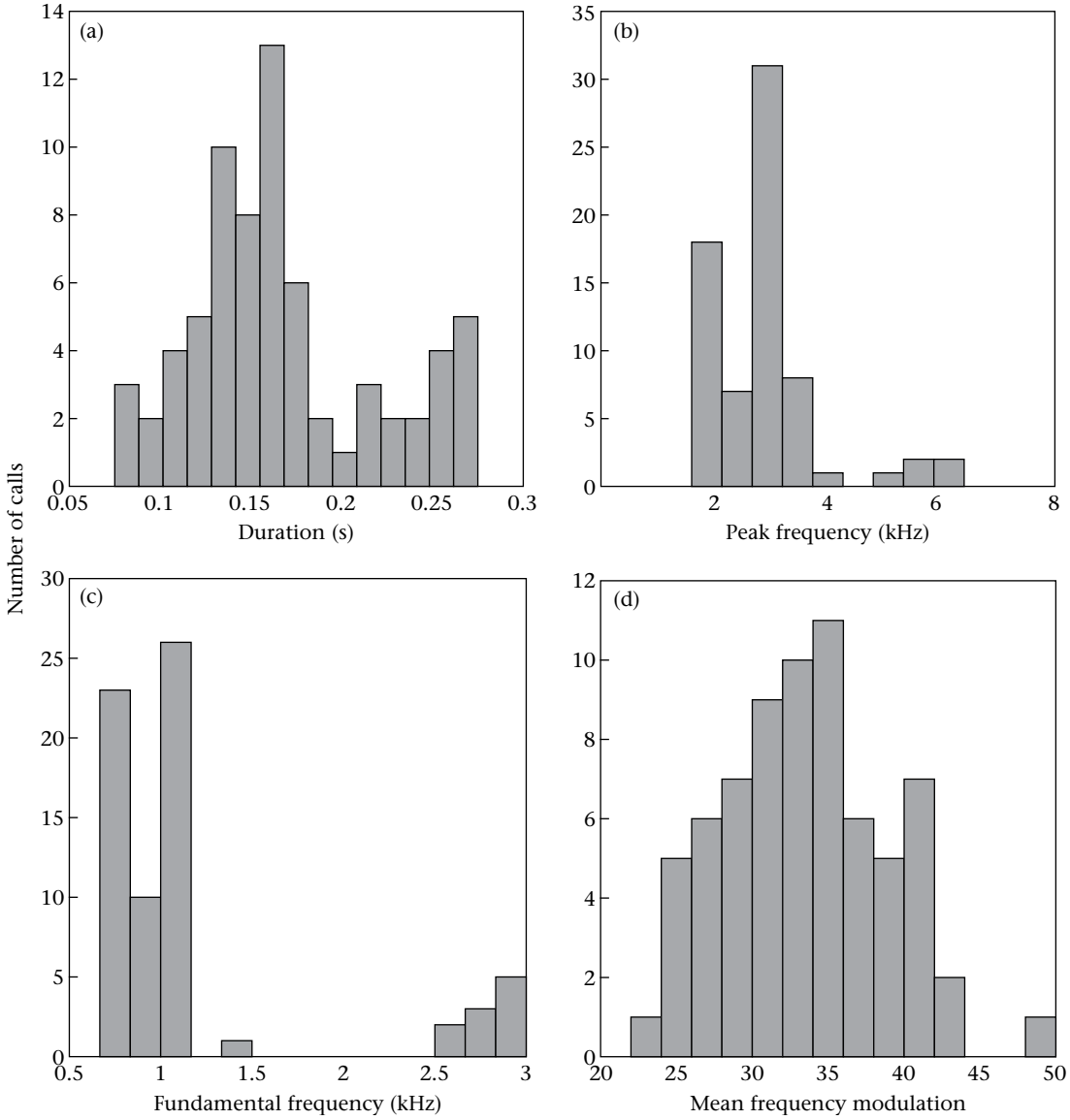

Figure 4. Histograms of acoustic variables describing contact calls from the western slopes region of the *Platycercus elegans* ring species complex in southeastern Australia, where crimson rosellas and yellow rosellas occur in sympatry. (a) Call duration, (b) peak frequency, (c) fundamental frequency and (d) mean frequency modulation. Frequency indicates the number of calls (1 or 2 calls per individual, $N = 70$ calls from 38 individuals; see text for details).

species hypothesis, CR and YR calls did differ significantly in mean FM, with AR and WS being somewhat intermediate, but crucially it does not appear that this divergence was maintained in the zone of sympatry (represented by WS populations). WS did not show significantly different mean FM from either CR or YR, and the distribution of mean FM in WS populations was unimodal (Fig. 4d). The DFA correctly classified only 55.3% and 52.0% of CR and YR, respectively, which was the lowest of the four populations and suggests that these populations do not have highly distinctive contact calls.

Few other putative ring species have been studied extensively, particularly in terms of vocalizations. In the greenish warbler, *Phylloscopus trochiloides*, complex, data on vocal variation appear to support the ring species model, since vocal structure changes gradually along independent axes along either side of the ring (Irwin 2000, 2001b, 2008). In addition, the terminal forms seem to have maximally distinct calls and songs even in sympatry, such that neither population recognizes the song of the other where they meet according to results from playback experiments. In contrast, analysis of song in the great tit, *Parus major*, complex led Päckert et al. (2005) to reject the ring species model. In this species, song features fell into two large geographical blocks that were acoustically homogeneous, rather than differing gradually around the ring. Furthermore, songs did not clearly diverge most between the terminal forms, and in sympatry only a mixed song and song from one of the terminal forms was observed. In both species, however, the pattern of vocal variation seems to be consistent with population history inferred from molecular data. It will now be interesting to compare vocal variation with population genetic variation (Joseph et al. 2008) to see how well rosella vocalizations correspond with their population history. Furthermore, as the ability for vocal learning is characteristic in both the oscine songbirds (which include greenish warblers and great tits) and parrots (Kroodsma 1982; Streidter 1994), rosellas offer a valuable, evolutionarily independent comparison with these taxa with regard to how vocalizations may vary between populations in vocal learners, and how culturally transmitted traits and population divergence are linked (Slabbekoorn & Smith 2002a).

Table 2
Comparisons of four acoustic variables describing contact calls from five zones of the Adelaide rosella in South Australia

Explanatory term	Mean	Estimate±SE	*F*	*df*	*p*
Duration (s)					
Zone					
Fleurieu Peninsula	0.159±0.008	0.016±0.035	7.085	4, 96	<0.001
Adelaide Hills	0.176±0.006	0.028±0.032			
East Mount Lofty Ranges	0.091±0.015	−0.048±0.033			
Clare Valley	0.154±0.016	0.023±0.034			
Flinders Ranges	0.143±0.021				
Site					
Fleurieu Peninsula (4)	0.148–0.176	<0.001–0.028	3.932	9, 96	<0.001
Adelaide Hills (5)	0.137–0.219	−0.022–0.060			
East Mount Lofty Ranges (2)	0.083–0.099	0.016±0.029			
Clare Valley (1)	NA				
Flinders Ranges (2)	0.131–0.155	0.024±0.041			
Date		<0.001±<0.001	4.220	1, 93	0.043
Peak frequency (kHz)					
Zone					
Fleurieu Peninsula	4.170±0.203	0.604±0.862	3.881	4, 99	0.006
Adelaide Hills	3.631±0.145	−0.276±0.8			
East Mount Lofty Ranges	3.041±0.381	−0.551±0.839			
Clare Valley	2.696±0.422	−0.803±0.847			
Flinders Ranges	3.185±0.517				
Site					
Fleurieu Peninsula (4)	3.512–4.562	−0.591–0.459	1.205	9, 99	0.301
Adelaide Hills (5)	3.141–4.352	−0.081–1.129			
East Mount Lofty Ranges (2)	2.947–3.134	0.186±0.721			
Clare Valley (1)	NA				
Flinders Ranges (2)	2.872–3.499	−0.627±1.029			
Date		0.002±0.004	0.280	1, 95	0.598
Fundamental frequency (kHz)					
Zone					
Fleurieu Peninsula	2.819±0.066	−0.144±0.282	64.435	4, 98	<0.001
Adelaide Hills	2.729±0.047	−0.271±0.262			
East Mount Lofty Ranges	0.806±0.186	−1.98±0.274			
Clare Valley	1.024±0.136	−1.719±0.276			
Flinders Ranges	2.790±0.169				
Site					
Fleurieu Peninsula (4)	2.596–3.055	−0.003–0.456	3.655	9, 99	0.001
Adelaide Hills (5)	2.389–2.965	−0.083–0.493			
East Mount Lofty Ranges (2)	0.763–0.849	0.087±0.362			
Clare Valley (1)	NA				
Flinders Ranges (2)	2.743–2.838	0.096±0.336			
Date		0.004±0.001	11.280	1, 96	0.001
Mean frequency modulation (kHz)					
Zone					
Fleurieu Peninsula	35.578±0.937	−7.715±4.000	5.824	4, 102	<0.001
Adelaide Hills	32.664±0.666	−8.346±3.712			
East Mount Lofty Ranges	39.316±1.765	−3.891±3.888			
Clare Valley	37.950±1.930	−3.761±3.916			
Flinders Ranges	38.929±2.397				
Site					
Fleurieu Peninsula (4)	33.997–38.753	0.415–4.755	5.817	9, 102	<0.001
Adelaide Hills (5)	26.846–41.076	−6.520–7.710			
East Mount Lofty Ranges (2)	37.820–40.812	2.991±3.340			
Clare Valley (1)	NA				
Flinders Ranges (2)	36.146–41.712	−5.566±4.770			
Date		0.041±0.018	4.973	1, 98	0.028

One or two consecutive calls per individual were analysed. Data were analysed using mixed models, which included the three terms listed and individual identity (and a constant). Means are estimated marginal means derived from these models for each level of the terms zone and site. Means and estimates are presented with SE for zone, or as a range for the sites within each zone with more than two sites; the estimate for the last level in each group was excluded and not shown as it is redundant and set to 0. Degrees of freedom are rounded to the nearest whole number. The number of sites is indicated in parentheses.

Vocal Variation in Parrots

Variation in the calls of wild parrots has been reported for several species (Saunders 1983; Wright 1996; Farabaugh et al. 1998; Baker 2000; Bradbury et al. 2001; Bond & Diamond 2005; Kleeman & Gilardi 2005). Not unexpectedly for lifelong vocal learners for whom vocalizations are thought to have important social functions (Farabaugh & Dooling 1996; Wright 1996; Bradbury 2003; Vehrencamp et al. 2003; Pepperberg 2004; Wanker et al. 2005), studies suggest that such variation may frequently occur on small geographical scales through cultural effects (e.g. on the level of roosts or local populations; Wright 1996; Farabaugh et al. 1998; Bradbury et al. 2001; Bond & Diamond 2005). This was also evident in our observations; we found significant variation in all acoustic variables at the levels of recording site and zones within AR, and the magnitude of these effects was often considerable (Tables 1, 2). With DFA, three of the five zones of AR were correctly classified with more than 70% accuracy. In our study, the intraclass correlation, which represents the variability in each acoustic variable associated with differences between individuals, was also particularly high for fundamental frequency ($\rho = 0.91$ across all populations, 0.81 in AR) and particularly low for peak frequency ($\rho = 0.41$ across all populations, 0.25 in AR). Further research will be required to identify precisely the factors modulating vocal variation at these levels in *P. elegans*. Our results underline the importance of wide geographical sampling and the multilevel analytical approach that we used for reliably discerning geographically broad patterns of variation such as those most relevant to the ring species hypothesis.

A further striking observation from our results was that calls from WS populations had dramatically smaller fundamental frequencies than those of most other populations (fundamental frequency is also represented by DF1 in Figs 3e, 5e), and were not intermediate between the calls of the adjacent CR and YR populations (Fig. 3c). Accordingly, DFA showed strongest discriminability of WS populations (84.2% correct classification), which were maximally separated by the discriminant function dominated by fundamental frequency (DF1: Fig. 3e). Similarly, although the statistical model predicts that AR populations overall have a value for fundamental frequency that is intermediate between those of WS populations and CR/YR populations (Fig. 3c), this outcome is driven by only two zones within AR. These two zones were Clare Valley (zone 3) and East Mount Lofty Ranges (zone 5). Contact calls from zones 3 and 5 feature significantly lower fundamental frequencies (Fig. 5c) which are comparable to those of WS populations, and good discrimination from other AR zones (zero and 12.5% incorrectly classified to other zones within AR, respectively; Fig. 5e).

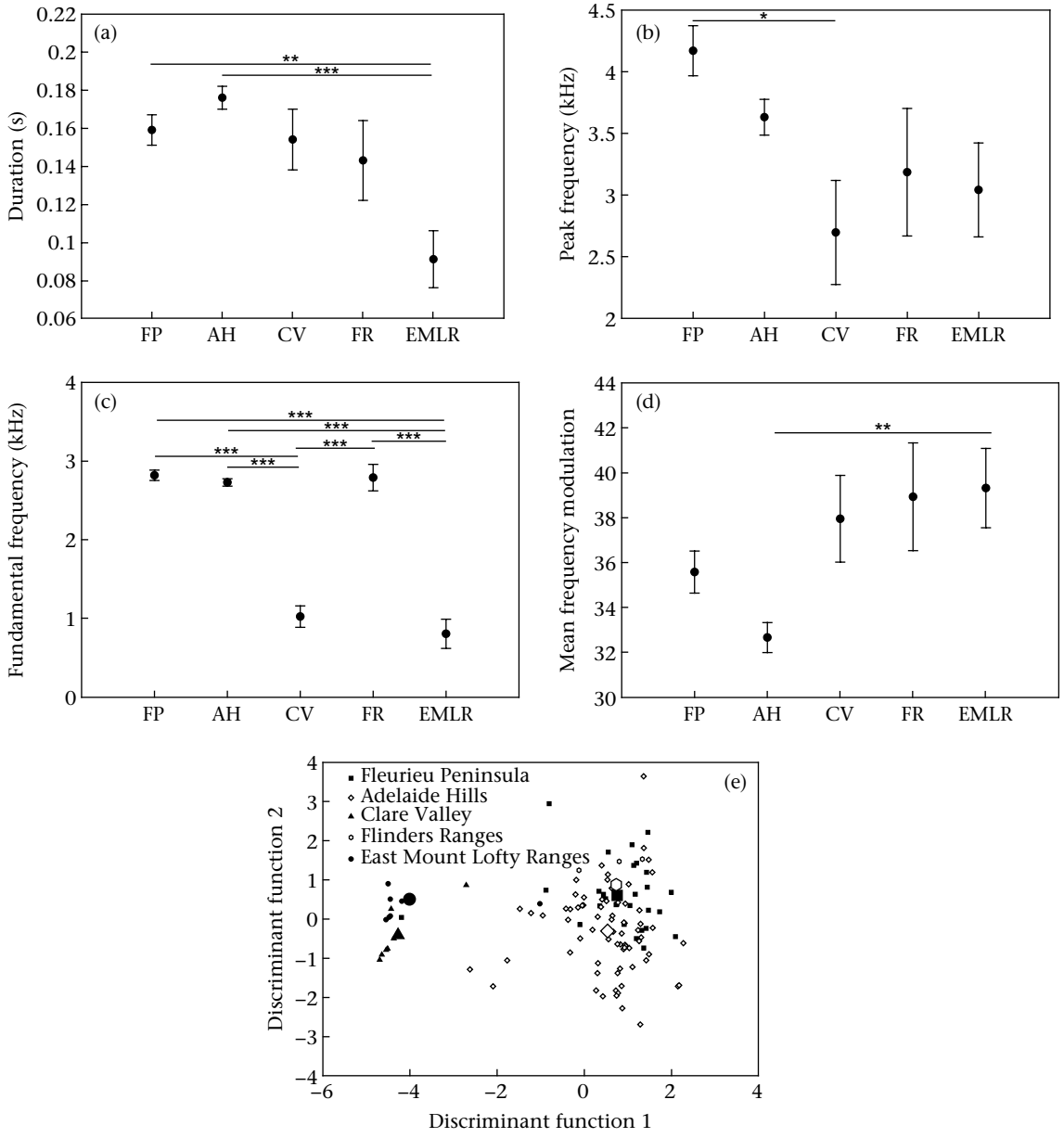

Figure 5. Acoustic variables describing contact calls from five zones of the Adelaide rosella: Fleurieu Peninsula (FP), Adelaide Hills (AH), Clare Valley (CV), Flinders Ranges (FR) and East Mount Lofty Ranges (EMLR). (a) Call duration, in which significant or nearly significant differences were found between East Mount Lofty Ranges and the Fleurieu Peninsula (mean difference ± SE = −0.07 ± 0.02 s (95% confidence interval, CI −0.12 to −0.02), $df = 93$, $P = 0.002$), Adelaide Hills (mean difference ± SE = −0.08 ± 0.02 s (95% CI −0.13 to −0.04), $df = 94$, $P < 0.001$) and Clare Valley zones (mean difference ± SE = −0.06 ± 0.02 s (95% CI −0.13 to 0.003), $df = 97$, $P = 0.075$). (b) Peak frequency, in which the Clare Valley zone was significantly lower than the Fleurieu Peninsula zone (mean difference ± SE = −1.48 ± 0.47 kHz (95% CI −2.81 to −0.14), $df = 106$, $P = 0.020$). (c) Fundamental frequency, in which we found significant differences between the East Mount Lofty Ranges zone and Fleurieu Peninsula (mean difference ± SE = −2.01 ± 0.20 kHz (95% CI −2.58 to −1.45), $df = 94$, $P < 0.001$), Adelaide Hills (mean difference ± SE = −1.92 ± 0.19 kHz (95% CI −2.47 to −1.37), $df = 94$, $P < 0.001$) and Flinders Ranges zones (mean difference ± SE = −1.98 ± 0.25 kHz (95% CI −2.70 to −1.27), $df = 93$, $P < 0.001$). The Clare Valley zone also had a significantly lower fundamental frequency than the Fleurieu Peninsula (mean difference ± SE = −1.80 ± 0.15 kHz (95% CI −2.23 to −1.36), $df = 105$, $P < 0.001$), Adelaide Hills (mean difference ± SE = −1.71 ± 0.14 kHz (95% CI −2.12 to −1.29), $df = 106$, $P < 0.001$) and Flinders Ranges zones (mean difference ± SE = −1.77 ± 0.22 kHz (95% CI −2.40 to −1.14), $df = 98$, $P < 0.001$). (d) Mean frequency modulation (FM), in which the East Mount Lofty Ranges zone and the Adelaide Hills zone were significantly different (mean difference ± SE = 6.65 ± 1.90 (95% CI 1.19 to 12.11), $df = 99$, $P = 0.007$). Bars show mean ± SE fitted values derived from multilevel models controlling for recording site, date and individual identity (see text and Table 2 for details). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (e) Discriminant function analysis of contact calls based on the four acoustic variables; each point represents an individual and the five large symbols indicate centroids for the five zones. Number of individuals: Fleurieu Peninsula: $N = 28$; Adelaide Hills: $N = 70$; Clare Valley: $N = 7$; Flinders Ranges: $N = 4$; East Mount Lofty Ranges: $N = 10$.

The conspicuous geographical differences in fundamental frequency that we observed raise the question, what has happened or is happening in these areas? Previous studies have attempted to understand frequency variation in avian vocalizations on both proximate and ultimate levels. For example, morphology, ontogeny, environmental variation and adaptation to acoustic windows have all been implicated in such variation (e.g. Morton 1975; Ryan & Brenowitz 1985; Podos 2001; Slabbekoorn & Peet 2003; Bond & Diamond 2005; Cynx et al. 2005; Riede et al. 2006). Ecological factors (reviewed in Slabbekoorn & Smith 2002a) are an unlikely explanation for our results, although they cannot be ruled out completely, since most of the habitat in which populations with low fundamental frequencies occur is intermediate to that of YR and CR populations, and populations with low fundamental

frequencies seem to be morphologically intermediate (Higgins 1999; Forshaw 2002). WS populations occur in a steep environmental cline between the hilly, forested habitat of CR and the much drier riparian woodland habitat of YR. Similarly, the East Mount Lofty Ranges zone of AR comprises a sharp, topographically steep interface between the wooded Mount Lofty Ranges occupied by the rest of AR and the lower, more open YR habitat. Ongoing genetic analyses allude to some degree of historical allopatry of AR, CR and YR populations, and indicate steeply stepped gene flow in the western slopes region and where YR approaches AR (Joseph et al. 2008) despite the predominance of morphologically intermediate individuals in these areas (Joseph & Hope 1984; personal observations). These regions are therefore likely to represent zones of secondary contact. This raises the prospect that sympatric interactions in such zones, such as reinforcement and character displacement, or drift resulting from limited migration into or out of these areas, may underpin the distinct call structure in WS and some AR populations. Under this scenario, other call differences between AR, CR and YR populations may have evolved to a large extent in allopatry, rather than through isolation by distance as predicted by the ring species hypothesis. Playback experiments, comparisons of vocal variation with genetic divergence, and analysis of the link between rosella call variation and fitness are needed to provide further insight into these possibilities.

Conclusions

In general, the links between culturally transmitted traits, population divergence and speciation remain controversial and poorly understood (Slabbekoorn & Smith 2002a; Haavie et al. 2004; Edwards et al. 2005). We found considerable geographical variation in the contact calls of *P. elegans*, on both small and large geographical scales. However, we conclude that the ring species concept, at least in the classical form, does not provide an adequate explanation for the pattern of geographical variation in these calls, at least based on the acoustic parameters that we analysed. We found that vocal variation was largely discordant with the previously described morphological variation between populations. This may partly reflect unique processes operating on vocal diversity. Furthermore, the underlying complexity of many long-held ring species may be relevant as many such species have probably arisen from more complicated population histories than previously realized. Some cases reflect periods of allopatric divergence and multiple zones of secondary contact, and these are now being revealed through molecular studies (Liebers et al. 2004; Päckert et al. 2005; Joseph et al. 2008). Our current results suggest that instances of secondary contact may be particularly important in promoting variation in vocal learners. These new, more rigorous insights should continue to make such species valuable targets for research into population divergence and speciation processes.

Acknowledgments

We thank Tom Bradley, Stephen Eustace, Jennifer Hoyal Cuthill, David Humphreys, Claire Newton, Thijs van Overveld, Leonie Raijmakers and Joe Woodgate for invaluable help in the field. For much helpful advice, we thank Jack Bradbury, Peter Bird, Steven Cooper, Steve Donnellan, John Endler, Bob Henzel, Elsie Krebs, Rob Magrath, David Paton, Maya Penck, Hans Slabbekoorn, Ron Sinclair and Richard Zann for access to the unpublished Postgraduate Diploma thesis of Robyn Astley. We owe special thanks to Brian and Maggie Colton (Uraidla), Bob and Jennie Henzell, Nelson and Kathy MacIntosh ('Mac's Land') for facilitating our fieldwork. We are grateful to many other landowners and managers who allowed us to work on their properties or who generously provided us with accommodation, particularly G. Ashton (Carey Gully), D. Hann ('Yabtree West'), M. Matheson (Sevenhill), and the Austin (Mundarlo), Barton (Bonegilla), Collins ('Billabong'), Edwards (Oakbank), Graves ('Yulti Wirra'), Laucke (Cambrai), McKendrick (Barnawartha North), Paynter (Quorn) and Piper (Brungle) families. We are also grateful to the staff of Deep Creek Conservation Park for their assistance. This project was funded by BBSRC grant B01568 to A.T.D.B. and K.L.B., a Leverhulme Trust Fellowship to A.T.D.B., a LESARS Studentship, University of Bristol Postgraduate Research Scholarship and an ASAB undergraduate project grant to R.F.H.R., the Marco Polo Fund (University of Groningen) and Deakin University. We are also grateful to the University of Adelaide for a visiting research Fellowship to A.T.D.B. and a visiting studentship to R.F.H.R.

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